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Dr.Joshua Lederberg
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Madison 6,Wisconsin

Dear Dr.Lederberg:

Thank you very much for your letter and for the most interesting reprints which you have kindly sent me. May I inquire whether you have any plans of going to Italy this Summer and participate at the Congress of Genetics and/or Microbiology? It would be certainly a very great pleasure to be able to meet you there personally, as I shall probably attend the genetical congress. Of course, it would be still more wonderful if you could visit Sweden at the same time...

We are just finishing a paper on some quantitative studies on the growth of two established ascites tumors. At various intervals after inoculation of various cell doses we have determined a).the amount of peritoneal fluid, b).the total number of free tumor cells, and c).the total number of non-tumorous exudate cells. It appears that there is a certain amount of peritoneal fluid present in the normal,untreated mouse; we call it V_o and its amount in mice of constant weight,strain and sex is about 0.12 ml. This fluid contains quite a number of non-tumorous cells (n-cells) the number of which we call e_o. When we inject a certain number of ascites tumor cells (T-cells),they intermix with the available fluid and also provoke the production of some new fluid. Surprisingly enough,we find that the amount of peritoneal fluid after inoculation, and,as a matter of fact, at any given time during ascites tumor growth can be adequately described by the following simple expression:

$$V = p.T + V_o$$

and the number of n-cells at any given time is

$$e = k.T + e_o$$

where V is the amount of peritoneal fluid at a certain point of time, T is the number of T-cells and e is the number of n-cells at the same time, p and k are constants and V_o and e_o are as defined above.

If we inject a comparatively small number of T-cells, which is still above the critical level needed for the production of ascites tumors in 100 per cent of the inoculated animals, the product p.T and k.T,respectively,is negligible compared to V_o and e_o in the above equations, and one may say that the cells inoculated are being simply diluted by the available peritoneal fluid and its content of n-cells. And still,the cells grow readily as free cells within this fluid, and the amount of fluid increases appreciably only after the number of cells has increased so much that the product pT is no longer negligible (the value of p is about 130×10^{-6}.).

This would mean that a provocation of new exudate by the injected cells is not a necessary condition for their growth, as they grow perfectly well in the fluid that is available - but, and this just what surprises us too to a great extent, only until the inoculum dose is not being decreased below the "critical level" which is usually of the order of 10^5 T-cells. If inoculum dose is being decreased further, an increasing percentage of the animals develop only solid tumors, usually at the site of inoculation. This means that a much smaller number of T-cells can already survive and multiply in the solid tissues of the host than in the peritoneal fluid. Another observation is that the "lag phase" (defined as the time needed before the number of free T-cells increases to a value significantly higher than the dose of inoculum) is inversely related to the number of inoculated cells in the case of inoculum doses above the critical level. In analogy with Hinshelwood's theory to explain bacterial lag, we have tried to speculate that there might be some active substance which is being contributed by the inoculated cells and which has to reach a certain critical concentration in the peritoneal fluid before the cells can start to grow. (I dont know how much Hinshelwood's theory is accepted for the case of bacterial lag, I must confess.) The peritoneal fluid is continuously circulating, and some experiments of Prentice et al. with tritium-labeled water have shown that as much as 40 to 80 per cent is being exchanged during one hour in case of much larger volumes than what we are dealing with. One could imagine that this continuous circulation necessitates the inoculation of at least 10^5 cells, otherwise the hypothetical active product becomes too diluted and never reaches the necessary critical concentration. Circulation of tissue fluid in solid tissues, where the tumor cells inoculated are crowded together, might be slower so as to enable the cells to build up the critical concentration of the active substance. All this is pure speculation and we try to design experiments to get closer to the problem. Your suggestions are certainly most welcome at any time.

The cultivation of ascites tumor cells in vitro, preferably in the form of suspensions, is very desirable indeed. We have made some very preliminary experiments several years ago, but have given it up, realizing the rather enormous technical difficulties. What we did was to try to grow them in their own ascites serum, diluted and supplied with embryo extract, and this was a complete failure. I tried to convince Dr. Earle at the National Cancer Institute that this is just the problem which he should solve. I dont think he was very convinced, although he wrote me some time ago that they will undertake some preliminary studies. I think he has the greatest experience and best organization to solve the question. However, if he looses interest, we are decided to try it again.

I am sure you will be more careful in the future to write me a letter when you get such a long and tedious answer. There is a great number of problems I should very much like to discuss with you in connection with the working hypothesis on a "mutation-selection" origin of ascites tumors. I hope that the opportunity will come in the not too far future.

With kindest regards,

very sincerely yours,

George Klein

P.S. Did your paper on genetic exchange in Salmonella appear yet? If reprints are available, I should very much appreciate receiving one.